Publication number: CN 1306003A

Title

Oligosaccharides used in drugs and health care products with the properties of anti-tumor activity and immunity enhancing

Abstract

The present invention relates to the structures of oligosaccharides with the properties of anti-tumor activity and immunity enhancing, and the structures of oligosaccharides used in the health care products or drugs for enhancing immunity, anti-tumor activity and treatment of cancer. The tests for the immunity and the anti-tumor activity show that these oligosaccharides are all gluco-oligosaccharides with 1→3β connections in the main chain and 1→6 or other connections in the side chain.

Claims

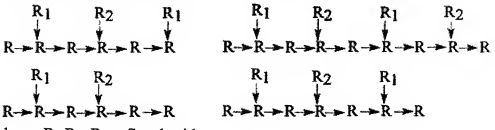
1. A structure of oligosaccharides with the properties of anti-tumor activity and immunity enhancing, and a structure of oligosaccharides used in the health care products or drugs for enhancing immunity, anti-tumor activity and treatment of cancer, wherein :

(1) the oligosaccharide have tetrasaccharide or pentasaccharide in the main chain: R→R→R→R R→R→R→R→R R = Saccharide

(2) the main chain in the oligosaccharides has two side chains;

where, R, R_1, R_2 = Saccharide

(3) bigger oligosaccharides contain the said basic structure of the main chain and the side chains (such as the oligosaccharides made of octasaccharide to tetradecasaccharide). Examples of the structure are shown as follows:



where, R, R_1, R_2 = Saccharide

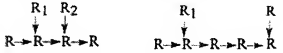
2. The structure of oligosaccharides of Claim 1 (1), wherein said main chain of tetrasaccharide or pentasaccharide are made of uniform glucoses, that is the main chain has the structure of Glu→Glu→Glu→Glu or Glu→Glu→Glu→Glu→Glu, where, Glu = Glucose

3. The structure of oligosaccharides of Claim 1 (1), wherein said main chain are formed through 1→3β connections, that is the main chain has the connections of β-Glu-1→3-β-Glu-1→3-β-Glu-1→3-Glu or β-Glu-1→3-β-Glu-1→3-β-Glu-1→3-β-Glu-1→3-Glu.

4. The structure of oligosaccharides of Claim 1 (1), (2) and (3), wherein said oligosaccharides are free oligosaccharides (with the reduction group of hydroxyl); alkyl, aryl glucosides of the oligosaccharides; or peptides of the oligosaccharides.

5. The structure of oligosaccharides of Claim 1 (2), wherein the side chains R1 and R2 are monosaccharides or oligosaccharides.

6. The structure of oligosaccharides of Claim 1 (2), wherein the side chains are attached to the main chain side by side closely or separated by the glucose units on the main chain. Examples of the structure are shown as follows:



7. The structure of oligosaccharides of Claim 1 (2), wherein the saccharides in the side chains are glucoses or other saccharides.

8. The structure of oligosaccharides of Claim 1 (2), wherein the saccharides in the side chains are connected to the main chain through 1→6β or 1→6α connections.

9. The structure of oligosaccharides of Claim 1 (2), wherein the saccharides in each side chain are connected to each other through any connections.

10. An usage of the oligosaccharides of Claim 1, separately or combined with other components through injection or oral medication, in the health care products or drugs for enhancing immunity, anti-tumor activity and treatment of cancer.

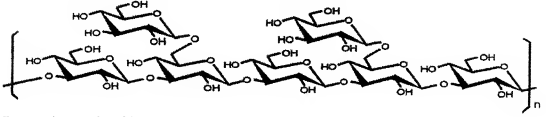
Description

Oligosaccharides used in drugs and health care products with the properties of anti-tumor activity and immunity enhancing

The present invention relates to the oligosaccharides with the properties of anti-tumor activity and immunity enhancing, especially to the oligosaccharides used in the health care products or drugs for enhancing immunity, anti-tumor activity and treatment of cancer.

As far as the research discovered recently, most of the saccharides with the properties of immunity enhancing and anti-tumor activity are oligosaccharides, such as lentinan, lucidum polysaccharide, which are extracted from domestic fungus or Chinese medicinal herbs. There are very few oligosaccharides which have been clearly researched and confirmed the functions of immunity enhancing and anti-tumor activity, for example, the α-L-Fuc(1-2)-β-D-Gal(1-3)-β-D-GalNAc-(1-3)-α-D-Gal-(1-4)-β-D-Gal-(1-4)-β-D-Glu (a heptasaccharide made of fucose, galactose, N-acetyl galactose and glucose), is a heptasaccharide antigen for curing mammary cancer (*J.Am.Chem.Soc.,117,1995,7840*), which is under clinic research now. Lentinan is an oligosaccharide with clearly confirmed property of immunity enhancing and anti-tumor activity, it can kill the cancer cells by enhancing the immunity of the human body but not attacking the cancer cells directly, and it is still unclear now that which receptor combines with the lentinan first when it comes into effect. The Japanese scientists, as the first discoverers of the anti-tumor activity property of lentinan, believe that the triple helix structure of lentinan is the key structure to keep the effectiveness of lentinan, and an example is given that the untreated lentinan with the molecular weight in millions as well as the smaller lentinan with the molecular weight of 14 thousands after acid hydrolysis all have the triple helix structure, thus they have similar effects (*Carbohydr.Res.1993, 245, 81-96. Nature 1969, 222, 687*). However, we believe that the triple helix structure of lentinan is made of the first level structure that the structure of the repeating unit of oligosaccharide building up the polysaccharide, and the oligosaccharides can react with receptors to enable the immunization. We have first disclosed the synthesis of the core fragment of lentinan in a former patent (Jun Ning, Fanzuo Kong, Chinese patent application number: 99126224.7), and the present invention is related to the research of the immunity enhancing and anti-tumor activity property of the oligosaccharide with the core fragment of lentinan and other oligosaccharides used in the health products or drugs for immunity enhancing, anti-tumor activity and treatment of cancer.

The structure of the repeating unit of heptasaccharide in lentinan is shown as follows.



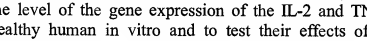
Repeating unit of lentinan heptasaccharide

The purpose of this invention is to confirm the structure of oligosaccharides with the properties of anti-tumor activity and immunity enhancing, and the structure of oligosaccharides used in the health care products or drugs for immunity enhancing, anti-tumor activity and treatment of cancer.

The purpose of this invention can be obtained through the following approach: test the effects of the following saccharides with the synthesized core fragment of lentinan on the level of the gene expression of the IL-2 and TNF-α mononuclear blood cells of a healthy human in vitro and to test their effects of anti-tumor activity: trisaccharide, tetrasaccharide, pentasaccharide, hexasaccharide, heptasaccharide, octasaccharide, nonasaccharide, decasaccharide, hendecasaccharide, dodecasaccharide, tridecasaccharide, tetradecasaccharide and the similar saccharides of these.

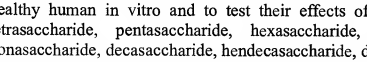
Based on these experiments, the oligosaccharides with the property of anti-tumor activity and immunity enhancing and thus used in the health care products or drugs for immunity enhancing, anti-tumor activity and treatment of cancer, have been confirmed to have the following structure:

(1) The main chain of the oligosaccharide is tetrasaccharide or pentasaccharide.



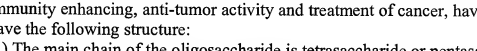
where, R = Saccharide

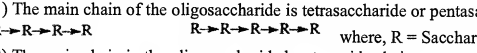
(2) The main chain in the oligosaccharide has two side chains.



where, R, R_1, R_2 = Saccharide

(3) Bigger oligosaccharides contain the said basic structure of the main chain and the side chains (such as the oligosaccharides made of octasaccharide to tetradecasaccharide). Examples of the structure are shown as follows:





where, R, R_1, R_2 = Saccharide

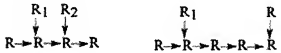
The said main chain of tetrasaccharide or pentasaccharide are made of uniform glucoses, that is the main chain has the structure of Glu→Glu→Glu→Glu or Glu→Glu→Glu→Glu→Glu, where, Glu = glucose

The said main chain are formed through 1→3β connections, that is the main chain has the connections of β-Glu-1→3-β-Glu-1→3-β-Glu-1→3-Glu or β-Glu-1→3-β-Glu-1→3-β-Glu-1→3-β-Glu-1→3-β-Glu-1→3-Glu.

The said oligosaccharides are free oligosaccharides (with the reduction group of hydroxyl); alkyl, aryl glucosides of the oligosaccharides; or peptides of the oligosaccharides.

The side chains R_1 and R_2 of the said oligosaccharides are monosaccharides or oligosaccharides.

The side chains are attached to the main chain of the oligosaccharides side by side closely or separated by the glucose units on the main chain. Examples of the structure are shown as follows:



The saccharides in the side chains of the said oligosaccharide are glucoses or other saccharides.

The saccharides in the side chains of the said oligosaccharide are connected to the main chain through 1→6β or 1→6α connections.

The saccharides in each side chain of the said oligosaccharides are connected to each other through any connections.

The said oligosaccharides can be used through injection or oral medication (separately or combined with other components). They can be used in the health care products or drugs for enhancing immunity, anti-tumor activity and treatment of cancer.

The present invention is further described in detail in the examples below.

1. The said oligosaccharides have obvious effects on the level of the gene expression and the activity of the IL-2 and TNF-α mononuclear blood cells of a healthy human in vitro, the levels of these genes' expression and their activities increase with the increase of oligosaccharides' concentration in the range of 0.5ml/liter to 15 ml/liter, so that to induce immunologic reaction.
2. The inhibition of tumor caused by the said oligosaccharides
   Anticancer effects and inhibition of metastasis of cancer cells caused by the said oligosaccharides

Cancer	Host	Dosage of the oligosaccharides (1 mg/kg × d)	Pathway	Injection time	Tumor inhibition rate of the oligosaccharides	Tumor complete-regression rate
Sarcoma 180	CD-I/JCR	1×10	i.p.	1 to 10	100	10/10
	A/J	5×4	i.p.	1 to 4	96.5	9/10
	DBA/2N	5×4	i.p.	1 to 4	100	10/10
	SWM/Ms	1×10	i.p.	1 to 10	100	10/10
Ehrlich peritoneal carcinoma	CD-I/ICR	1×10	i.p.	1 to 10	54.7	0/5
CCM adenocarcinoma	SWM/Ms	1×10	i.p.	1 to 10	65.3	0/10
MC.SI fibrosarcoma	A/J	1×10	i.p.	1 to 10	100	18/18
MC.CSI fibrosarcoma	DBA/2N	1×10	i.p.	1 to 10	76.5	2/7
MM-46 carcinoma	C3H/HeN	5×2	i.v.	13,15	100	9/9
M-I09 carcinoma	BALB/c	25×2	i.p.	15,18	91	8/22
MC-primary carcinoma	DBA/2N	1×10	i.p.	1 to 10	80.5	2/5
MH,134 hepatic carcinoma	C3H/HeN	1×14	i.p.	21 to 40	100	10/14
Methyl cholanthrene	SWM/Ms	1×10	i.p.	21 to 31	83→33%	
Methyl cholanthrene	DBA/2N	1×10	i.p.	14 to 24	78→37%	
Adenosis type12	C3H/HeN	3×10	i.p.	14 to 18	79→40%	

Therefore, the said oligosaccharides can obviously inhibit the growth of similar transplanted cancers as S180 cancer, as well as the syngenic cancer and idio-cancer, and have prevention on the chemical carcinogenesis and the virus carcinogenesis.

The inhibition of tumor caused by the said oligosaccharides has the characteristics that: the effectiveness depends on the host rather than the cytotoxicity of tumor cells; There is an optimal dosage, over-dose will weaken the effects of anti-tumor activity and immunity enhancing; the effectiveness varies obviously on different mice species. Mice of the species of A/J, DBA/2, CD-1 et. al. are the most sensitive ones to the said

oligosaccharides, there shows complete regression of tumor in those mice. There shows no regression of tumor in the mice of the C3H/He species, and medium level of regression of tumor in the mice of the specie of BALB/C, CBA et. al.. Inoculate methyl cholanthrene (MC) to the sensitive mice to create fibrosarcoma cell strains, and transplant the tumor to the mice of the inbred line, then there shows a regression of the tumor after administration of the said oligosaccharides.

The said oligosaccharides have strong inhibition on the chemical carcinogenesis. Almost all the mice grow tumor after the transplant of MC for 35 weeks, however, the cancerigenic probability reduces to about 30% if the said oligosaccharide is taken via abdominal cavity after the transplant of MC for 2 to 3 weeks. This is because that the small amount of cancer cells initiated by the transplant of MC is killed by the enhanced immunity via the injection of the said oligosaccharides. The said oligosaccharides can also reduce the cancerigenic probability of adenovirus from 80% to 40%. This indicates that the said oligosaccharides are not only the agent to enhance the immunologic rejection but also the true anticancer agent.

Moreover, the said oligosaccharides also have great effects on the infection of various bacterial, virus and parasites.